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TITLE: CHARACTERIZATION OF ROUTINE MICROBORE LIQUID CHROMATOGRAPHY IN THE TOBACCO INDUSTRY

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ABSTRACT: Recent trends in liquid chromatography have emphasized small columns and fast analyses. New microbore LC columns (I.D. ~1 mm) offer advantages in reduced solvent consumption and increased sensitivity. Reduced solvent consumption and increased solute sensitivity are related to the ratio of the square of the internal diameters of the analytical scale and microbore columns. In a general approximation, this results in a 90-98% mobile phase reduction and a 20-fold increase in sensitivity. This paper describes the applicability of Whatman® Micro-B LC columns for routine use in a tobacco laboratory. Practical characterization of the Whatman Micro-B column includes studies which determine the optimum sample injection volume, column capacity, the optimum flowrate, and the optimum detector volume. Several simple modifications of standard Waters® liquid chromatography equipment which produce the flowrates appropriate for a microbore column will be presented. Waters systems operating at these reduced flowrates have been utilized to perform tobacco-related separations. One such application is glycyrrhizic acid separation from licorice. The precision of ten replicate 1- l injections by the Waters Wisp automatic sampler injector was 1.5% relative standard deviation (RSD) by peak area and 1.4% RSD for peak height when quantitating glycyrrhizic acid.

REVIEW: Introductory remarks were made in which the general properties of microbore LC were covered. Normal HPLC packings were used (5-10 u), in 1-mm ID columns. The equipment used by the authors consisted of a Waters 6000A pump; 660 solvent programmer; Whatman Partisil ODS-3 microbore column, 1-mm ID x 25 cm, 5u; Waters WISP autosampler; Waters 441 fixed wavelength UV detector; and 0.007" ID microbore SS interconnecting tubing for injector/column/detector connections. The data was handled by a CALS in-house computer system. A flow rate of 30 ul/minute was utilized. The objective was to apply microbore techniques to ordinary analytical problems, for which conventional HPLC methods exist. An RP test mixture was used for preliminary measurements of several variables. This mixture contained compounds with various functional groups. When injection volumes were varied from 1-14 ul, efficiency was drastically lower over 3 ul. When the connecting tubing length was varied (5-50 cm), loss of efficiency due to 50 cm of tubing was only ~3%. Variation in flow cell volume from 2 1 to 12 ul gave a 2-15% loss in efficiency, depending on the K' of the compound used for the measurement, with less effect occurring at higher K'. Modification of a 12-ul cell by inserting teflon tubing to achieve a 2-ul volume gave 90-100% efficiency compared to a commercial 2-ul cell. The system was adapted to the analysis of licorice extract. No significant change in performance resulted from 150 injections. Precision was good. Some loss of efficiency was observed relative to conventional HPLC (Waters RCSS or Whatman ODS-3 columns), but it was not more than 15%. This had a very small effect on resolution. Best efficiency is realized at lower K' (4-5) than in conventional HPLC. The savings in solvent consumption is ~95% compared to 4.6 mm ID columns.

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